SUBSTITUTE SPECIFICATION

CHELATING AGENTS FOR RADIOIMMUNOTHERAPY, AND PROCESS FOR PREPARING THE SAME

The invention relates to compounds useful as chelating agents, complexes between said compounds and radioelements, and to their uses, in particular in pharmaceutical compositions and compositions for the diagnosis of pathologies such as cancers.

Immunotherapy with radiolabeled antibodies should allow fairly specific targeting of certain cancers (Schubiger et al., 1996; Parker, 1990). However, iodine-131 (Bardies et al., 1992; Stein et al., 1995) may not be the best isotope for tumor therapy because of its limited specific activity, low beta-energy, relatively long half-life and strong gamma emission.

Another approach to improving therapeutic efficacy is the use of replacement isotopes with better physical properties. Chelators that can hold radiometals with high stability under physiological conditions are essential to avoid excessive radiation damage to non-target cells. Moreover, the development of new bifunctional chelating agents is essential for this purpose. Thus synthesis of new chelating agents able to bind radiometals such as rhenium-188, yttrium-90, samarium-153 or Bismuth-213 and in general all the α and β particles emitters will be required to possess sufficiently stable chelators.

Accordingly, one of the aim of the invention is to provide chelating agents forming stable complexes *in vivo* with the numerous potential candidates for such applications.

The stability of a non-macrocyclic ligand can be favorably influenced by the preorganization of the open chain. In fact, a semi-rigid structure such as that of *trans* 1-2 diaminocyclohexane limits the rotation of the ethylene bridge, so that the purpose of the cyclohexane design is to preorient the four pendent arms in a skew position.

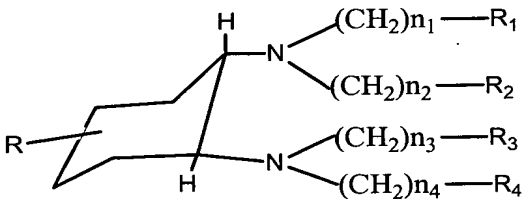
A first investigation (Mease et al., 1990), which was guided by a study performed on polyaminocarboxylic acid ligands incorporating the skeleton of ethylenediaminetetraacetic acid (EDTA) in a cyclohexane structure, showed the influence of this semi-rigid structure on the stability of the resulting complexes. A second study (Goeckeler et al., 1987) of the stability of lanthanides as ^{153}Sm-polyaminophosphonic acid complexes showed that ethylenediamine

tetramethylphosphonic acid (EDTMP) derivatives allow stable quantitative ^{153}Sm chelation.

The (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane dihydro chloride compound, the structural derivative of *trans*-1,2-diaminocyclohexane, have been prepared (Gestin et al., 1997; Loussouarn, et al., 1998). This intermediate, which is functionalized at position 4 of the cycle by a protected amine termination (Meares et al., 1984) for future covalent attachment to biomolecules, allows the introduction of different chelating groups via the free amines.

The Inventors have developed a new simple and efficient synthesis pathway from *trans* -1,2- diaminocyclohexane to provide access to a new class of semi-rigid chelating agents. This same reactional scheme applies to the reactional intermediary, (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane dihydrochloride, which allows the synthesis of these same chelating agents, though functionalized back of the cycle by a termination allowed coupling to an antibody or any other biological substance such as a hapten.

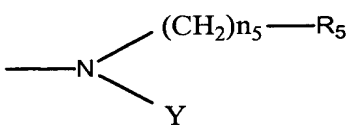
The present invention relates to compounds of the following formula (I) :



(I)

in which :

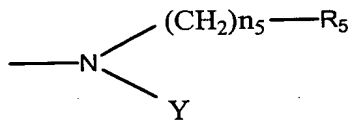
- n_1, n_2, n_3 and n_4, independently from each other, represent an integer from 1 to 5, preferably from 1 to 3,
- R_1, R_2, R_3 and R_4, independently from each other, represent :
  - . –COOH,
  - . –$PO(OH)_2$,



wherein n_5 represents an integer from 1 to 5, preferably from 1 to 3, R_5 represents –COOH or -$PO(OH)_2$, and Y represents H or a group –(CH2)n_6-R_6

in which n_6 represents an integer from 1 to 5, preferably from 1 to 3, and R_6 represents –COOH or -$PO(OH)_2$,

provided that at least one of R_1, R_2, R_3 or R_4 represents a group



such as defined above,

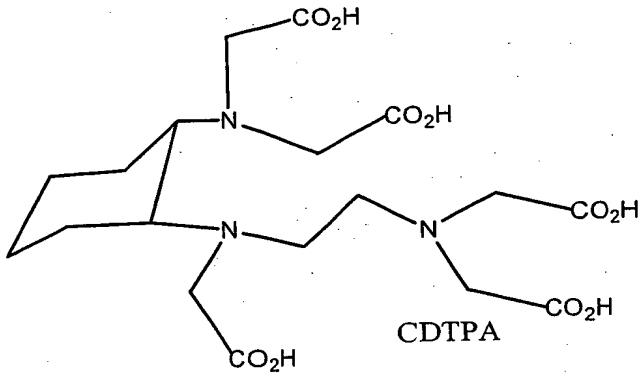
- R represents :

. H, or –$NHCOCH_3$, or

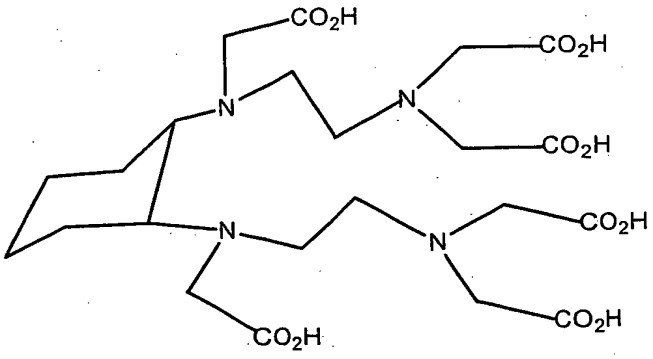
. a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, which are able to bind specifically with epitopes located at the surface of the cells of the organism, or to chemical or biological compounds located at the surface of a solid carrier, or

. a group carrying a function linked, if necessary via a binding site, to molecules as defined above,

the two following compounds, CDTPA and CTTHA, being excluded :



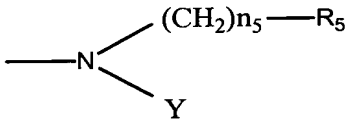
CDTPA



CTTHA

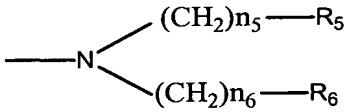
The invention relates more particularly to compounds of formula (I) such as defined above, characterized in that :

- when R_1, R_2, R_3 or R_4 represents -COOH or -$PO(OH)_2$, then n_1, n_2, n_3 or n_4 represents 1 respectively,
- when R_1, R_2, R_3 or R_4 represents a group



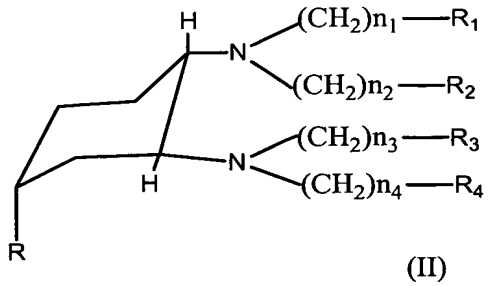
then n_1, n_2, n_3 or n_4 represents 2 or 3 respectively, and preferably 2,
- n_5, and optionally n_6, represents 1.

The invention also relates more particularly to compounds of formula (I) such as defined above, characterized in that at least one, and more preferably two of R_1, R_2, R_3 and R_4, represent a group



wherein n_5, n_6, R_5 and R_6 are defined above.

Preferred compounds of formula (I) such as defined above, wherein R is different from hydrogen, are compounds of the following formula (II) :



(II)

wherein n_1, n_2, n_3, n_4, R_1, R_2, R_3, R_4 and R are such as defined above.

The invention relates more particularly to compounds of formula (I) or (II) such as defined above, characterized in that R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined above, and in particular R represents a group chosen among all the coupling functions for vector or solid support binding, such as the following groups :

. alcohol group, such as -OH,
. amino group, such as $-NH_2$, $-NO_2$,
. aldehyde group, such as -CHO,
. carboxylic group, such as -COOH,
. anhydride group, such as -CO-O-CO-R",
. $-CO-CH_2X$, wherein X represents an halogen atom, such as Cl or Br,
. -CO-X, wherein X represents an halogen atom, such as Cl or Br,
. a diazonium ion N_2^+,
. an activated ester, such as -COOR", R" = ethyl or N-hydrosuccinimide,
. a sulfonic group, such as SO_3H,
. a thiocyanate group, such as -NCS, or an isocyanate -NCO, or a -NH-NCS group
. a thiol group, such as -SH,
. a disulfure group, such as -S-S-R".

The invention also concerns compounds of formula (I) or (II) such as defined above, characterized in that R represents a group carrying a function linked, if necessary via a binding site, to molecules, such as antibodies, haptens or peptides, as defined above, and more particularly R represents a group chosen among the following groups :

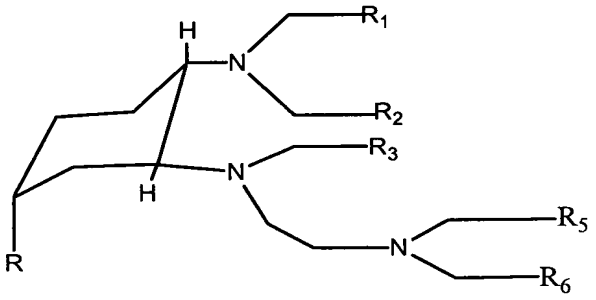
. -O-CO-R',
. -NH-CO-R',
. -NH-CS-R',
. -CH=N-R',
. -CO-NH-R',
. $-CO-CH_2$-NH-R',
. -N=N-NH-R',
. $-SO_2$-NH-R',
. -NH-CS-NH-R',
. -thioether-R',
. -CO-S-R',
. $-CO-CH_2$-S-R',
. -S-S-R',
. $-NH-CH_2$-R',

. -CO-NH-N=CH-R',

. -CS-NH-N=CH-R',

wherein R' represents said molecule.

The invention concerns more specifically compounds such as described above of the following formula (III) :



(III)

in which R_1, R_2, R_3, R_5 and R_6 independently from each other represent -COOH or -$PO(OH)_2$, and R is a group as defined above.

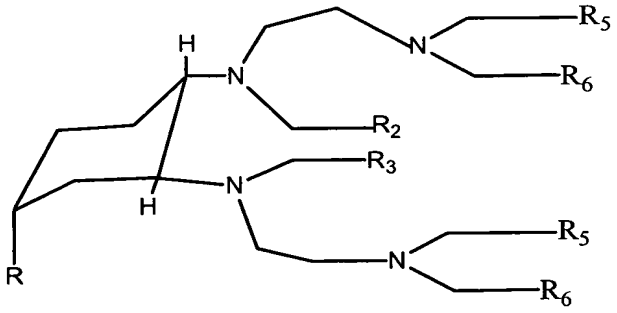
Preferred compounds of formula (III) are such that :

. $R_1 = R_5 = R_6$ = COOH and R_2, = R_3 = $PO(HO)_2$, or

. $R_1 = R_2 = R_3 = R_5 = R_6$ = COOH, or

. $R_1 = R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

The invention also concerns more specifically compounds such as described above, of the following formula (IV) :



(IV)

wherein R_2, R_5 and R_6, independently form each other, represent – COOH or -$PO(OH)_2$, and R is a group as defined above.

Preferred compounds of formula (IV) are such that :

. $R_2 = R_3 = PO(OH)_2$, and $R_5 = R_6$ = COOH, or

. $R_2 = R_3 = R_5 = R_6 = COOH$, or

. $R_2 = R_3 = R_5 = R_6 = PO(OH)_2$.

The invention also relates to complexes between a compound such as described above, and a radioactive element, said complexes resulting from the association of said radioelement with the -COOH and/or $-PO(OH)_2$ groups of said compound (the bonds between said radioelement and said compound being ionic bonds).

The above-mentioned radioelements are more particularly α, β or γ emitter radiometals, and preferably from the groups of actinides or lanthanides.

The invention relates more particularly to complexes such as described above, characterized in that said radioelements are α or β emitter radiometals (susceptible to be used in therapy, and more particularly in radioimmunotherapy in the frame of cancer treatments).

Advantageously, α emitter radiometals are chosen among the followings : Actinium 225, Bismuth 213.

Advantageously, β emitter radiometals are chosen among the followings : ^{33}P, ^{199}Au, ^{121}Sn, ^{177}Lu, ^{67}Cu, ^{105}Rh, ^{47}Sc, ^{77}As, ^{153}Sm, ^{159}Gd, ^{143}Pr, ^{186}Re, ^{111}Ag, ^{149}Pm, ^{109}Pd, ^{166}Ho, ^{32}P, ^{188}Re, ^{194}Ir, ^{142}Pr, ^{90}Y.

Preferred complexes with radiometals used in therapy, as defined above, are such that the compound is chosen among those wherein R represents a group carrying a function linked, if necessary via a binding site, to molecules as defined above, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,
- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step of the treatment) to epitopes on the surface of specific cells in the organism,
- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

The invention also concerns the use of a complex such as described above, for the manufacture of a medicament for radioimmunotherapy (also called radiopharmaceutical), in particular for the treatment of cancers, or for the treatment against metastase proliferation.

More particularly, the invention relates to the use of a complex such as defined above, for the manufacture of a medicament for the treatment of :

- lung cancer, said complex preferably being such that it comprises a radioelement chosen among : ^{188}Re, ^{186}Re, ^{153}Sm, ^{67}Cu and ^{90}Y, and wherein R comprises an antibody specific for lung cancer cells, such as Anti N-CAM Antibody, Anti CEA Antibody, Anti Carbohydrates Antibodies, or an hapten chosen among Anti N-CAM-679 Bispecific antibody, Anti CEA-679 Bispecific antibody, Anti Carbohydrates-679 Bispecific antibody, Anti N-CAM-734 Bispecific antibody, Anti CEA-734 Bispecific antibody, Anti Carbohydrates-734 Bispecific antibody,

- liver and pancreatic cancers, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for liver and pancreatic cancer cells, such as antibodies and haptens described above in the case of lung cancer,

- ovarian cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for ovarian cancer cells, such as OC125, MOV18, MOV19, OVTL3, or an hapten chosen among OC125-679 Bispecific antibody, MOV18-679 Bispecific antibody, MOV19-679 Bispecific antibody, OVTL3-679 Bispecific antibody, OC125-734 Bispecific antibody, MOV18-734 Bispecific antibody, MOV19-734 Bispecific antibody, OVTL3-734 Bispecific antibody,

- bladder cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for bladder cancer cells, such as AC48-127, or an hapten chosen among 48-127 Bispecific antibody, 48-127-679 Bispecific antibody, 48-127-734 Bispecific antibody,

- colorectal cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for colorectal cancer cells, such as Anti CEA Antibody, Anti Carbohydrates Antibodies, or an hapten chosen among Anti CEA-679 Bispecific antibody, Anti Carbohydrates-679 Bispecific antibody, Anti CEA-734 Bispecific antibody, Anti Carbohydrates-734 Bispecific antibody,

- thyroid medullary cancer, said complex preferably being such that it comprises a radioelement chosen among those cited above in the case of lung cancer, and wherein R comprises an antibody specific for thyroid medullary cancer cells, such as Anti CEA Antibody, or an hapten chosen among Anti CEA-679 Bispecific antibody, Anti CEA-734 Bispecific antibody,

- lymphoma, said complex preferably being such that it comprises a radioelement chosen among : ^{213}Bi, ^{225}Ac, ^{153}Sm, and ^{67}Cu, and wherein R comprises an antibody specific for lymphoma cells, such as specific antibody against expressed antigens surfaces lymphocyte cells, e.g. CD19, CD37, or an hapten such as bispecific antibody against expressed antigens surfaces lymphocyte cells, e.g. CD19-679, CD37-679, CD19-734, CD37-734,

- myeloma, said complex preferably being such that it comprises a radioelement chosen among : ^{213}Bi, ^{225}Ac, ^{153}Sm, and ^{67}Cu, and wherein R comprises an antibody specific for myeloma cells, such as specific antibody against expressed antigens surfaces myeloma cells, e.g. BB4, or an hapten such as bispecific antibody against expressed antigens surfaces myeloma cells, BB4-679, BB4-734,

- osteoarticular pathology, particularly in bone cancer extension balance.

The invention also concerns pharmaceutical compositions characterized in that they comprise an effective amount of a complex such as described above, in association with a suitable pharmaceutical carrier.

Pharmaceutical compositions according to the invention are more particularly characterized in that they are in a form suitable for an IV or IP administration in located areas.

Preferred pharmaceutical compositions according to the invention, are characterized in that the daily dosage is comprised between 1 and 100MBq /kg, e.g. between 3,7 and 74MBq/kg.

The invention also relates to complexes, as defined above, between a compound such as described above, and a radioactive element, characterized in that the radioelements are γ emitter radiometals (i.e. radiometals susceptible to be used in diagnosis methods, such as radioimmunoscintigraphy).

Advantageously, said radiometals are chosen among ^{111}In, ^{99m}Tc, ^{64}Cu.

Preferred complexes with radiometals used in diagnosis, as defined above, are such that the compound is chosen among those wherein R represents a group carrying a function linked, if necessary via a binding site, to molecules as defined above, and more particularly among those compounds wherein the group R comprises :

- an antibody (polyclonal or monoclonal) liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism,

- or an hapten, i.e. a non-immunogenic molecule of low MW capable of inducing the production of antibodies against itself, said hapten being liable to recognize and to bind to one or several molecules already bound (in a first step

of the method of diagnosis) to epitopes on the surface of specific cells in the organism,

- or a peptide resulting from the association of different amino acids and liable to recognize and to bind to specific epitopes on the surface of specific cells of the organism.

The invention also concerns the use of a complex such as described above, for carrying out diagnosis methods such as radioimmunoscintigraphy.

More particularly, the invention concerns the use of a complex such as defined above, for carrying out the following diagnosis methods by radioimmunoscintigraphy :

- diagnosis of cancers, such as cited above, the complex used being preferably such that it comprises ^{111}In, or ^{99m}Tc as radioelements, and R comprises an antibody or a hapten specific for such cancer cells, such as antibodies or haptens mentioned above in the frame of the cancers listed above,
- diagnosis of cardiovascular diseases, such as graft rejection, myocardic infarcts,
- diagnosis of cerebral diseases,
- diagnosis of renal diseases, in particular in the study of individual kidney functions, location of ectopic kidney, renal filtration and secretion troubles,
- vascular diseases, such as embolism and thrombosis, the complex used being preferably such that it comprises ^{111}In, or ^{99m}Tc as radioelements, and R comprises an antibody such as anti platelets or anti fibrin antibodies.

The invention also concerns the use of the complexes defined above for carrying out bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

The invention also relates to methods for the *in vitro* diagnosis of pathologies listed above, characterized in that it comprises the steps of incubating a biological sample, such as serum, plasma urines, with a complex as described above, the components of the biological sample being fixed to a solid carrier, rinsing the solid carrier and detecting the γ emission of the complex bound to the components of the sample on the solid carrier.

The invention also concerns the kits for carrying out said diagnosis methods, said kits comprising complexes as described above according to the invention.

The invention also relates to the use of a compound of formula (I) defined above, included compounds CDTPA and CTTHA, if necessary in the form of complexes with radiometals as defined above, for the manufacture of a

medicament useful as an analgesic, more particularly in the case of bone pathologies.

The invention also relates to the use of a compound of formula (I) defined above, included compounds CDTPA and CTTHA, for the manufacture of a medicament for the treatment of pathologies where ionic imbalances occur, or against the formation of stones in the organism.

The invention also relates to the use of a compound of formula (I) defined above, included compounds CDTPA and CTTHA, for carrying out a process for the detoxication of polluted medium, such as liquid phases polluted by bivalent or trivalent metals radioactives or not.

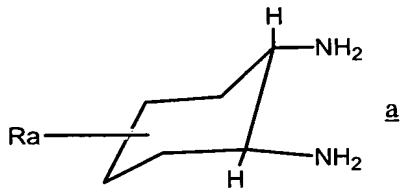
The invention also concerns a process for the detoxication of a polluted medium comprising the steps of contacting said medium with a compound as defined above, advantageously itself bound to a solid carrier, and recovering said medium substantially free of contaminants which are bound to said compound on the solid carrier.

The invention also relates to the use of a compound of formula (I) defined above, included compounds CDTPA and CTTHA, for carrying out a process for the radionuclides purification, said compound of the invention being bound to a solid phase.

The invention also relates to the use of a complex between of formula (I) defined above, included compounds CDTPA and CTTHA, for carrying out a bone scintigraphy, in particular in the frame of the diagnosis of osteoarticular pathology, particularly in bone cancer extension balance.

The invention also relates to processes for preparing compounds and complexes as described above. A process for the preparation of compounds according to the invention, comprises the following steps :

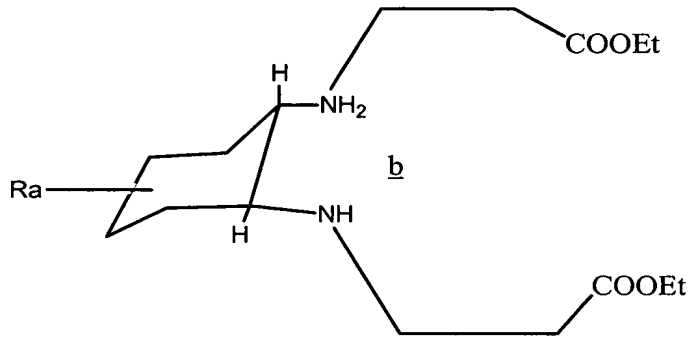
- contacting trans-1,2-diaminocyclohexane of the following formula a :



a

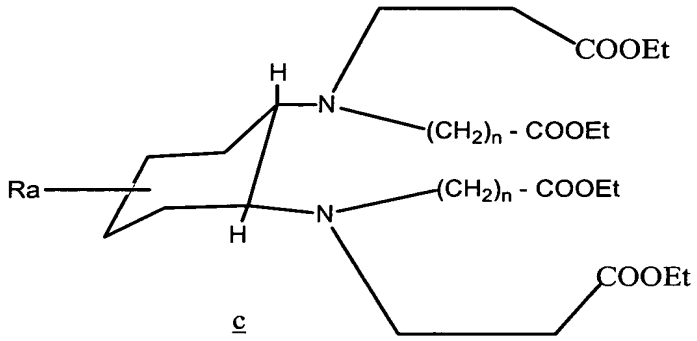
wherein R_a is H or $NHCOCH_3$,

* either with vinyl propionate, preferably by stirring 20h at room temperature, leading to the following compound b



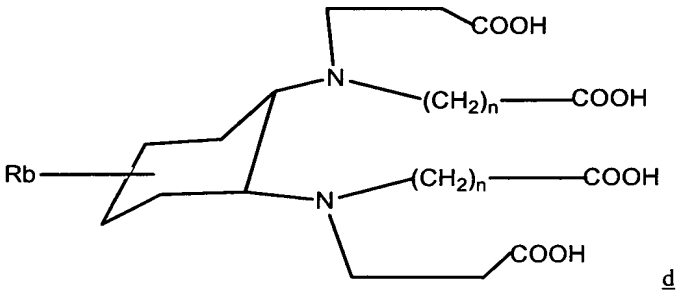
b

. contacting compound b with X-$(CH_2)_n$-COOEt, wherein X represents an halogen atom, and n represents an integer from 1 to 5, preferably at reflux during 15h, leading to the following compound c



c

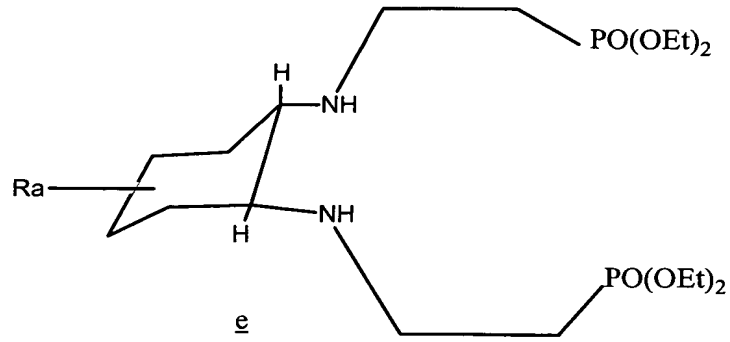
. treating compound c with HCl, preferably 6N HCl at reflux overnight, leading to the following compound d



d

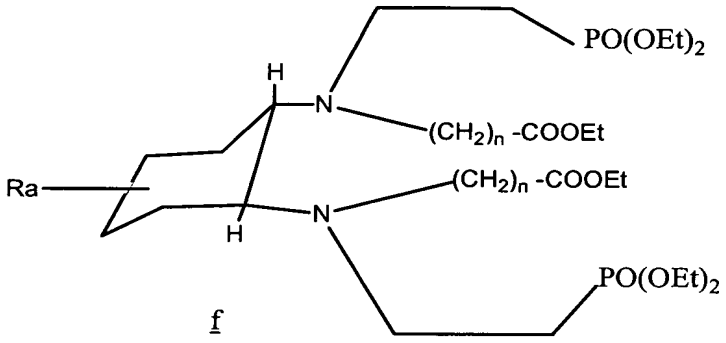
wherein R_b represents H or NH_2,

* or with diethyl vinyl phosphonate, preferably by stirring 15h at reflux, leading to the following compound e



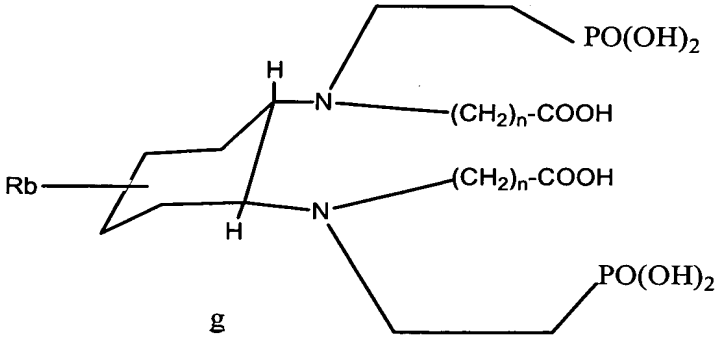
e

. contacting compound e with X-$(CH_2)_n$-COOEt, wherein X represents an halogen atom, and n represents an integer from 1 to 5, preferably at reflux during 15h, leading to the following compound f



f

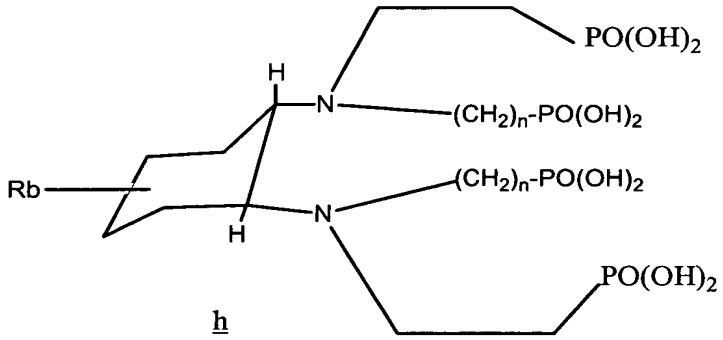
. treating compound f with HCl, preferably 6N HCl at reflux overnight, leading to the following compound g



g

wherein R_b represents H or NH_2,

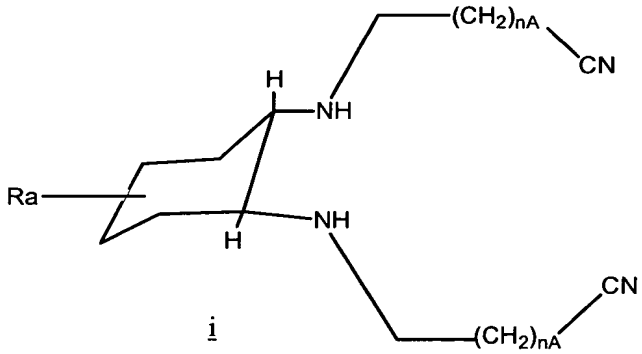
. if desired, treating compound g with phosphorous acid, preferably by stirring 30 mn at 80°C, leading to the following compound h



h

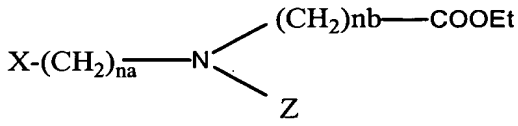
Another process for the preparation of compounds according to the invention, comprises the following steps :

- contacting the compound of formula a described above with a compound of formula H_2C=CH-$(CH_2)_{nA}$-CN wherein nA = 0 (acrylonitrile), or nA represents a integer from 1 to 3, preferably at room temperature during 20h, leading to the following compound i

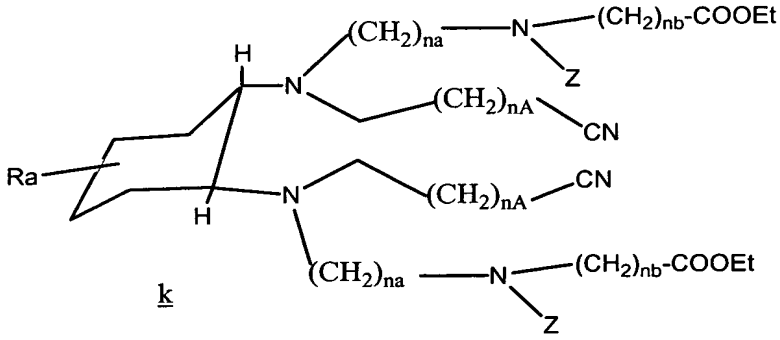


i

- contacting compound i with the following compound j

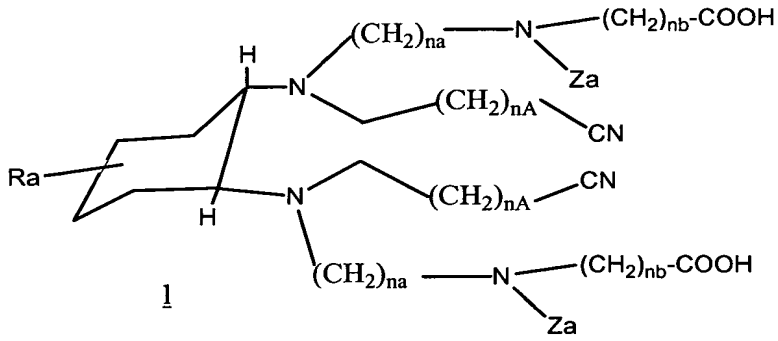


wherein X represents an halogen atom, na and nb, independently from each other represent an integer from 1 to 5, Z represents H or $(CH_2)_{nc}$-COOEt, and nc represents an integer from 1 to 5, preferably at 70°C during 2 days, leading to the following compound k



k

. treating compound k with HCl, preferably 6N HCl at reflux overnight, leading to the following compound l

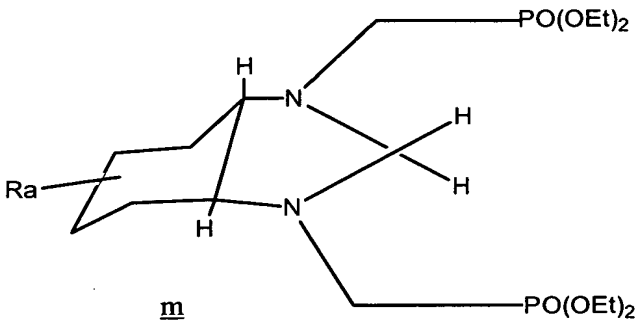


l

wherein Za represents H or -(CH2)nb-COOH, nA, na and nb being such as defined above, and R_b represents H or NH_2.

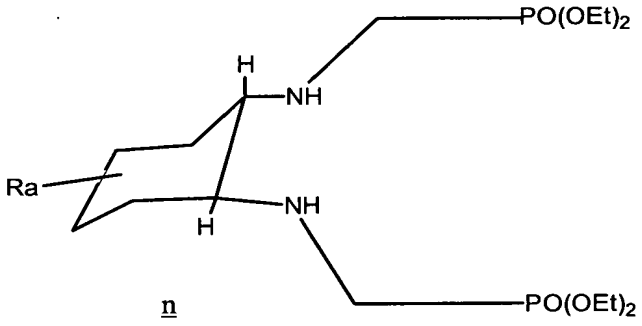
Another process for the preparation of compounds according to the invention, comprises the following steps :

- contacting the compound of formula a described above with paraformaldehyde and diethylphosphite, preferably in THF at reflux during 4h, leading to the following compound m



m

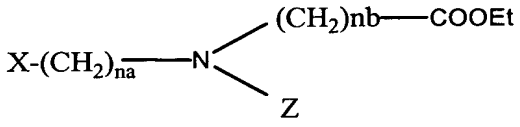
- treating compound m with HCl, preferably 3N HCl in MeOH at 50°C overnight, leading to the following compound n



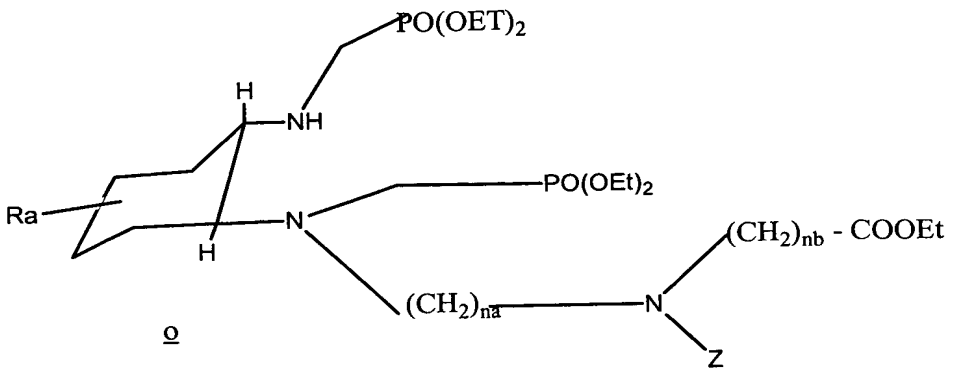
n

- contacting compound n :

* either with 1 equivalent of compound j

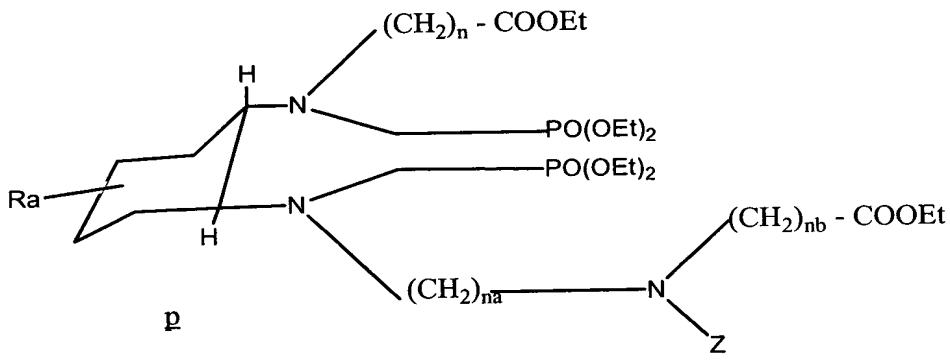


as described above, preferably at 70°C during 2 days, leading to the following compound of formula o



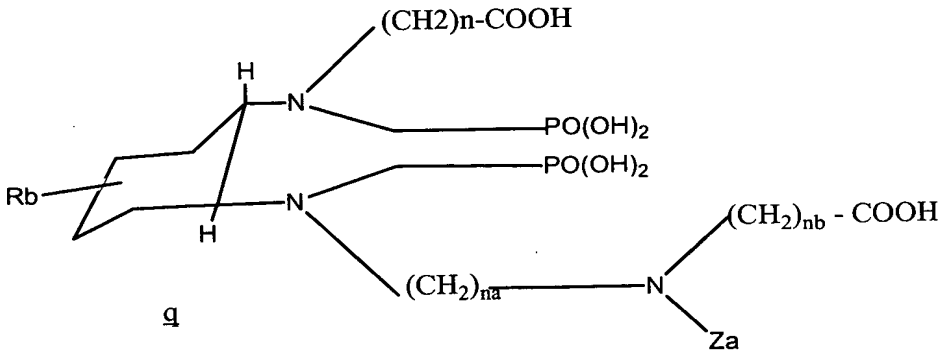
o

. contacting compound o with X-$(CH_2)_n$-COOEt, wherein X represents an halogen atom, and n represents an integer from 1 to 5, preferably at reflux during 15h, leading to the following compound p



p

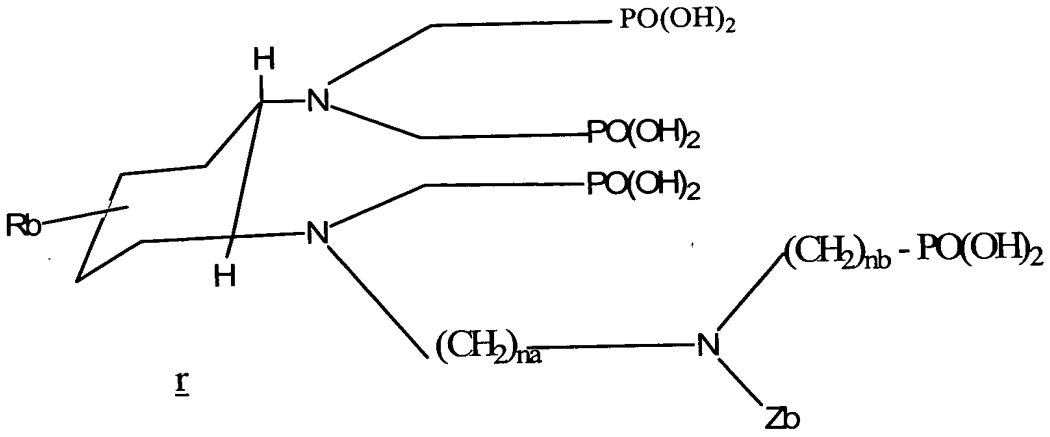
. treating compound p with HCl, preferably 6N HCl at reflux overnight, leading to the following compound q



q

wherein Za represents H or -$(CH_2)_{nb}$-COOH, na, nb and n being such as defined above, R_b represents H or NH_2,

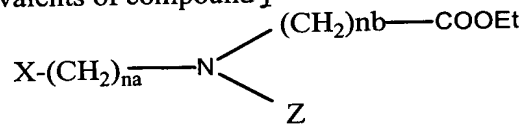
. if desired, treating compound q with phosphorous acid, preferably by stirring 30 mn at 80°C, leading to the following compound r



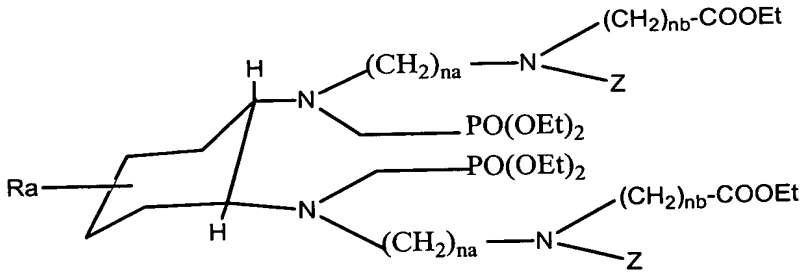
r

wherein Zb represents H or -$(CH_2)_{nb}$-$PO(OH)_2$

* or with 2 equivalents of compound j

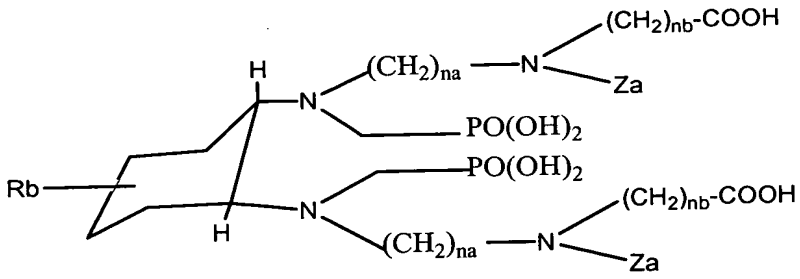


as described above, preferably at 70°C during 2 days, leading to the following compound of formula s



s

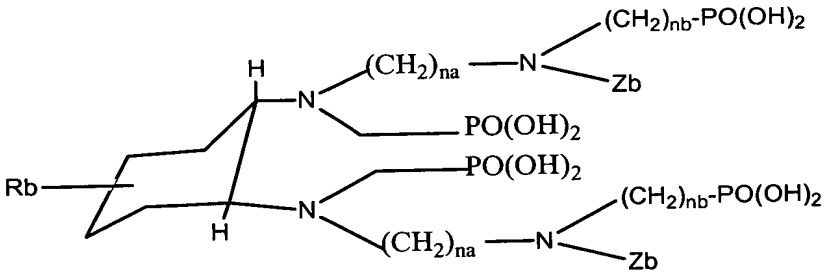
. treating compound s with HCl, preferably 6N HCl at reflux overnight, leading to the following compound t



t

wherein Za represents H or -$(CH_2)_{nb}$-COOH, na, nb and n being such as defined above, R_b represents H or NH_2,

. if desired, treating compound t with phosphorous acid, preferably by stirring 30 mn at 80°C, leading to the following compound u



u

wherein Zb represents H or -$(CH_2)_{nb}$-$PO(OH)_2$.

Compound of formula a can be obtained according to the method described in Gestin et al., 1997, and Loussouarn et al., 1998.

Compounds wherein Rb represents NH_2 obtained according to the processes described above, can then be transformed in order to correspond to compounds of formula (I) wherein R represents a group carrying a function liable to bind, if necessary via a binding site, to molecules as defined above.

By way of example, compounds of formula (I) wherein R represents -N=C=S, can be obtained by treatment of said compounds wherein Rb represents NH_2 with CSCl2, preferably in acidic or basic conditions.

Compounds of formula (I) wherein R represents a group carrying a function linked, if necessary via a binding site, to molecules as defined above, can then be obtained by coupling said compounds, wherein R represents a group carrying a function liable to bind to said molecules, with said molecules.

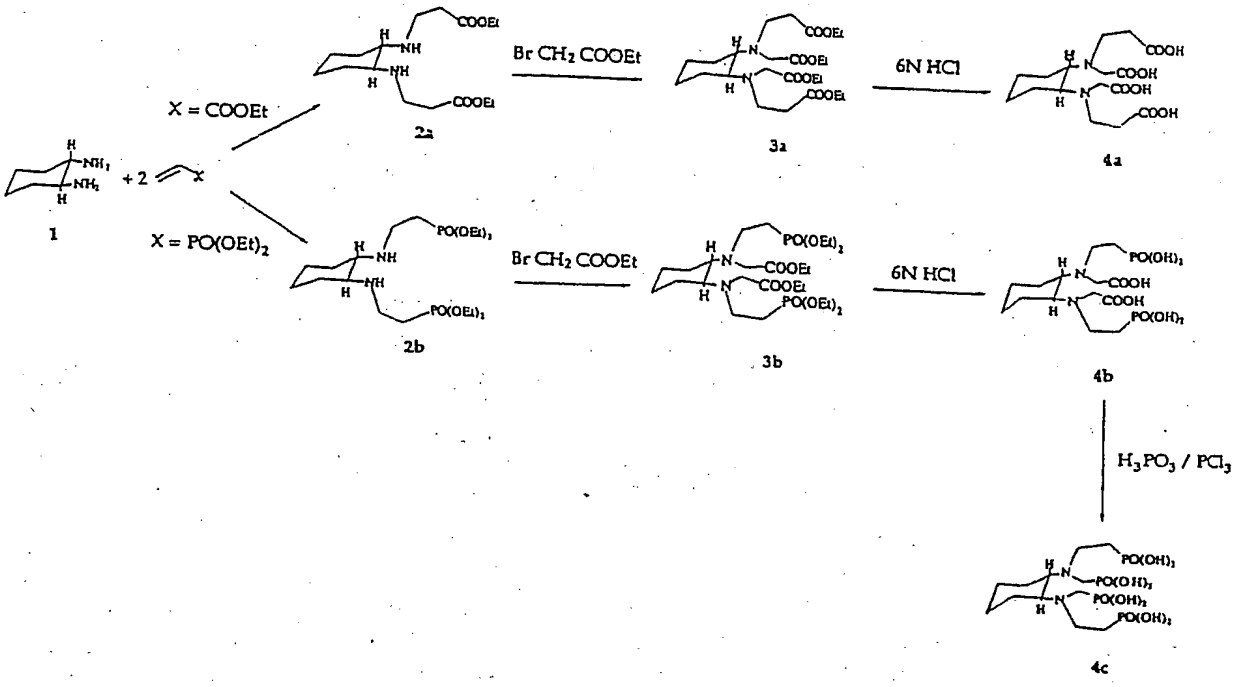
Complexes according to the invention are advantageously obtained by incubating the compounds with the radioelements at 37°C during 3 hours.

The invention will be further illustrated in the following examples for the preparation of compounds and complexes according to the invention.

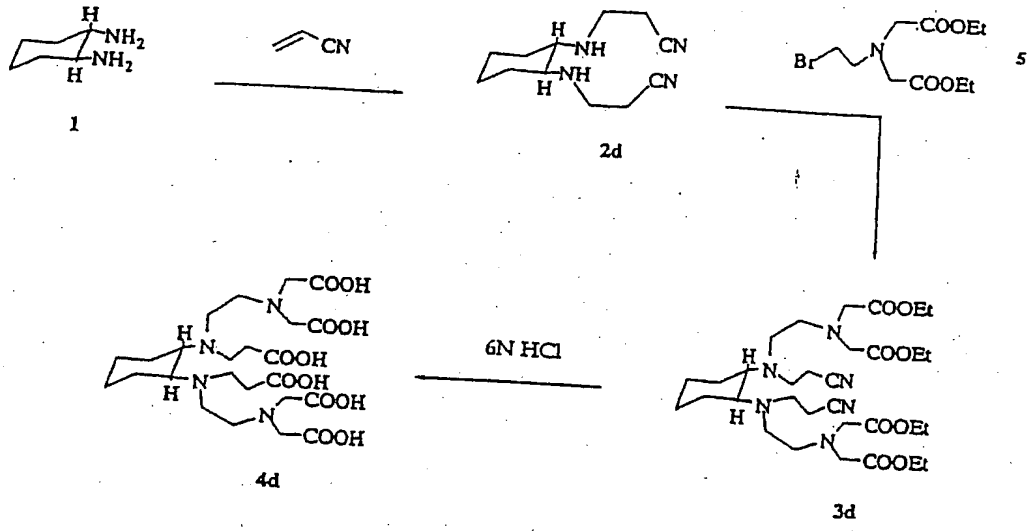
In order to save the functionalized reactional intermediate, i.e. the (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane dihydro chloride compound, the commercial product, *trans*-1,2- diaminocyclohexane **1**, has been used as starting material.

Two approach routes to differently substituted amines were carried out successfully.

- The first depicted in schemes I and II was the Michael type addition of primary amines to some vinylic derivatives to provide monoaddition with high selectivity (Bergeron et al., 1981), allowing N-alkylation to be envisaged at this step. In strategy depicted in scheme I, compounds **2a** and **2b** were alkylated by ethyl bromoacetate under conditions recommended by Studer (Studer and Meares, 1992) (KI and Na2CO3) to give tetraesters **3a** and **3b**. Acid-catalysed hydrolysis of the ester functions was performed in 3M hydrochloric acid to give the tetracarboxylic acid **4a** and the mixed acid **4b**. At last, in order to generate the structure **4c**, carboxylic functions were converted into phosphonic functions using H_3PO_3/PCl_3 according to the method of Krüger and Bauer (Krüger and Bauer, 1972). The other strategy described in scheme II required preparation of protected bis-carboxymethylated amino ethyl bromide. In view to convenience of deprotecting ethyl esters by acid-catalysed hydrolysis, *N,N-bis(*ethylacetate)-2-bromoethyl-amine was prepared according to the Williams and Rapoport's procedure (Williams and Rapoport, 1994) with minor modifications. N-alkylation of **2d** with the branching group in a mixed solvent system (CH_3CN/EtOH) at 70°C gave **3d** in 60% yield. Acid-catalysed hydrolysis of the ester functions as well as nitriles is the more convenient method (Ornstein et al., 1989), of hexacarboxylic acid **4d** preparation.

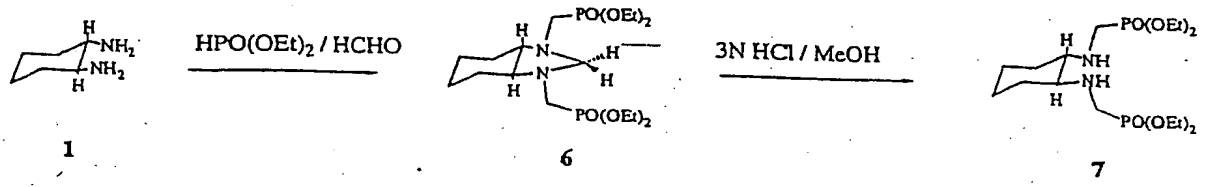


scheme I



scheme II

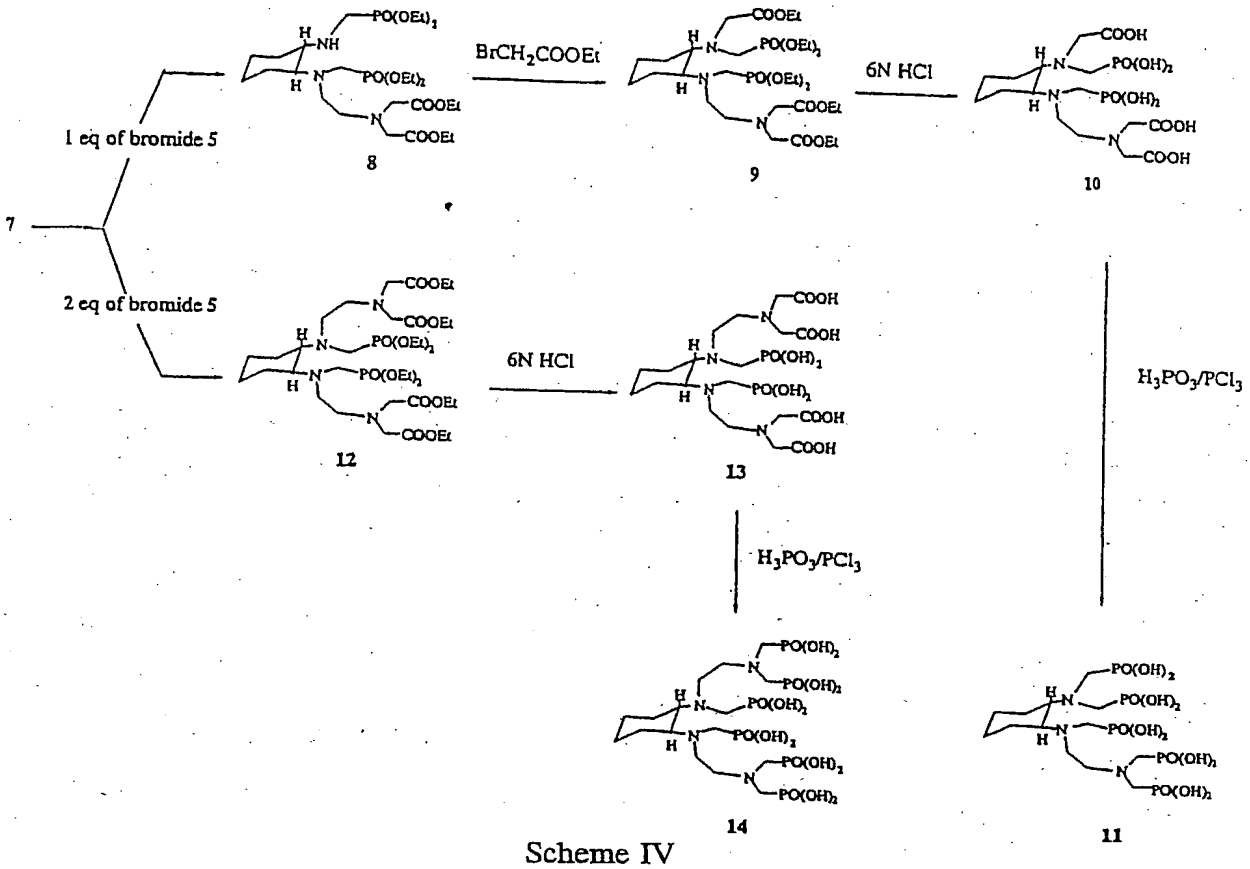
- In the case of certain diamines as *trans*-1,2-diaminocyclohexane **1**, the second route, as shown in schemes III, IV allowed the aminophosphonomethylation of amines protected by a methylene bridge between the two nitrogen atoms of **1**. This protecting group will subsequently provide for a different functionalization on the amine. The reaction of Kabachnick-field described and detailed by Baily and Burgada (Baily and Burgada, 1995), gave compound **6** which was prepared from paraformaldehyde and diethylphosphite in THF. **7** was obtained by removing the protecting group in acidic conditions.



1 **6** **7**

scheme III

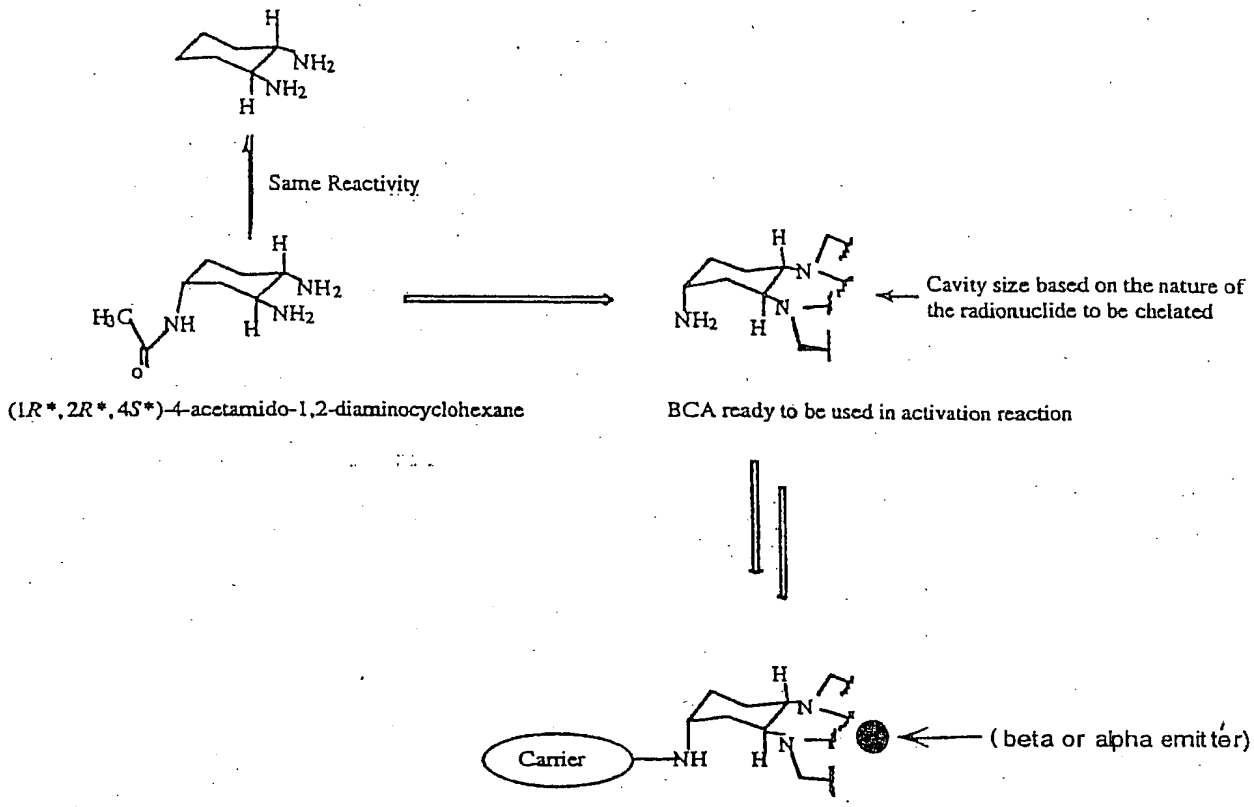
The monoalkylation or the dialkylation (see scheme IV), depending on the stoechiometry of the reaction gave respectively compounds **8** and **12**. The mixed acid **13** was obtained after hydrolysis of **12** in 6M hydrochloric acid and the hexaphosphonic acid **14** was prepared according to the method of Bauer and Kruger as described above. The synthesis of chelating agents **10** and **11** required an additional step which was the alkylation of **8** by ethyl bromoacetate to give the mixed ester **9**. Acid-catalysed hydrolysis gave **10** and **11** after reaction of conversion described all above.



Scheme IV

In conclusion, different non-functionalized ligands bearing aminophosphonate or aminocarboxylate chelate groups and mixed chelate groups were prepared and tested for their complexation properties with ^{153}Sm. The synthetic method described above was applied to the previously synthesized intermediate, the (1*R**, 2*R**, 4*S**)-4-acetamido-1,2-diaminocyclohexane, resulting in the synthesis of several polyaminocarboxylic acids, polyaminophosphonic acids and mixed semi-rigid functionalized ligands (BCA).

The different access routes to non-functionalized compounds described here were used without modifying the synthesis in order to obtain their functionalized homologues ready to be used in a coupling reaction as described in scheme V. We observed the influence of aminocarboxylic acid and aminophosphonic acid functions on the stability of the resulting complexes.



Scheme V

Experimental

General Procedures

All experiments were performed under nitrogen. Solvents were distilled prior to reactions. The primary chemicals used were commercial products (Sigma-Aldrich Company). Product purity and reaction progress were monitored on thin-layer chromatography (TLC) plates (60 F254, Merck), and liquid chromatography was carried out on a silica gel column (Merck 60,70-230 mesh). TLC revelation was performed under UV light (254 nm) or by iodine.

Nuclear Magnetic Resonance (MNR)

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AC 250 spectrometer (250 Mhz). Chemical shifts are reported in ppm to phosphoric acid as reference (85% H_3PO_4 in heavy water), positive values being downfield.

Chemical shifts (d) are reported in ppm. Coupling constant J is reported in Hertz (Hz).

Mass Spectrometry (MS)

MS spectra were recorded on a Mat Finnigan LCQ Ion Trap mass apparatus using the electrospray method in negative or positive mode.

Starting Material

The bisphosphonate **6** was prepared in our laboratory according the synthesis procedure of Baily and Burgada with minor modifications.

Synthesis and Specstroscopic data

***N,N'*-[(2-ethoxycarbonyl)eth-1-yl]-*trans*-cyclohexane-1,2-diamine 2a:** To freshly distilled *trans*-1,2-diaminocyclohexane 1 (1 ml, 8.33 mmol) in 50 ml of ethanol was added vinyl propionate (1.50 ml, 13.7 mmol) in one portion. After stirring 20h at room temperature, the reaction mixture was concentrated by rotary evaporation to yield a pale yellow oil (2.6 g, 8.32 mmol, 100%) witch was used directly in the next step. ^{1}H NMR ($CDCl_3$): d 1.22 (t, 12H), 1.67 (m, 2H), 1.82 (m, 2H), 2.06 (m, 2H+2H), 2.43 (t, 4H), 2.67 (dt, 2H), 2.98 (dt, 2H), 4.10 (q, 4H). ^{13}C NMR ($CDCl_3$): d 14.17, 24.31, 31.46, 35.34, 42.19, 60.23, 61.29, 172.69 . (M+H+): 315

***N,N'*-[(2-diethylphosphono)eth-1-yl]-*trans*-cyclohexane-1,2-diamine 2b:** To freshly distilled *trans*-1,2-diaminocyclohexane 1 (1 ml, 8.33 mmol) in 50 ml of ethanol was added diethyl vinyl phosphonate (2.80 ml, 18.21 mmol). The reaction mixture was allowed to stir at reflux during 15 hours. After removal the solvent under reduced pressure, the resulting oil was purified by column chromatography (silica gel, CH_2Cl_2-EtOH 1:1) to give 2.9 g of a limpid oil (6.65 mmol, 80%). ^{1}H NMR ($CDCl_3$): d 0.94 (m, 2H), 1.15 (m, 2H), 1.24 (t, 12H), 1.64 (m, 2H), 1.83-1.95 (m, 8H), 2.05 (m, 2H), 2.71 (dt, 2H), 2.97 (dt,

2H), 4.07 (dq, 8H). ^{13}C NMR ($CDCl_3$): d 16.37, 16.46, 25.42 (JC-P : 149 Hz), 28.20, 31.41, 40.47, 40.51, 61.29, 61.40, 61.50. (M+H+): 443

N,N'-[(2-cyano)eth-1-yl]-*trans*-cyclohexane-1,2-diamine **2d:** To freshly distilled *trans*-1,2-diaminocyclohexane 1 (1 ml, 8.33 mmol) in 50 ml of ethanol was added acrylonitryle (1.20 ml, 18.32 mmol). After stirring 20h at room temperature, the reaction mixture was concentrated by rotary evaporation to yield an pale yellow oil witch was purified by recristallisation in diethylether to give 1.40 g of a white solid (6.35 mmol, 78%): mp : 65°C

^{1}H NMR ($CDCl_3$): d 1.02 (m, 2H), 1.22 (m, 2H), 1.70-1.79 (m, 2H + 2H), 2.02-2.17 (m, 2H+2H), 2.49 (t, 4H), 2.80 (dt, 2H), 3.02 (dt, 2H). ^{13}C NMR ($CDCl_3$): d (M+H+): 221

N,N'-[(2-ethoxycarbonyl)eth-1-yl]-*N,N'*-(ethylacetate)-*trans*-cyclohexane-1,2-diamine **3a:** To a solution of 2a (1 g; 3,.18 mmol) in 50 ml of freshly distilled CH3CN under nitrogen were added Na_2CO_3 (0.50 g; 3.01 mmol) and KI (g; mmol). After stirring for 1 hour at 60°C, $BrCH_2COOEt$ (1.80 ml; 7.15 mmol) was added dropwise. The reaction mixture was kept at this temperature over a period of 24 hours prior to cooling to room temperature, filtration and concentration under reduced pressure. The residue was taken up in $CHCl_3$ (200 ml) and washed with water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow-brown oil. The crude product was purified by column chromatography (silica gel, CH_2Cl_2-EtOH 95:5). The fractions containing pure product were collected and dried to give an limpid oil (0.8 g; 1.56 mmol; 49 %). ^{1}H NMR ($CDCl_3$): d 1.10 (m, 4H), 1.26 (t, 12H), 1.91 (m, 2H), 2.00 (m, 2H), 2.51 (dt+m, 4H+2H(CH cycle)), 2.95 (dt, 4H), 3.39 (d, 4H), 4.12 (m, 8H). (M+H+): 515

N,N'-(ethylacetate)-*N,N'*-[(2-diethylphosphono)eth-1-yl]-*trans*-cyclohexane-1,2-diamine **3b:** The tetraester has been prepared as described above for compound from 1 g of compound 2b, Na_2CO_3 (0.70g, 6.60 mmol), KI (0.40, 2.40 mmol) and ethylbromoacetate (1.80 ml; 7.15 mmol). Purification by chromatography (SiO_2 (CH_2Cl_2-MeOH 99 : 1) gave 0.58 g of a pale yellow oil (0.94 mmol; 41%).^{1}H NMR ($CDCl_3$): d 1.11 (m, 4H), 1.25 (t, 6H), 1.29 (t, 12H), 1.70 (m, 2H), 1.85-2.10 (m, 4H+2H), 2.90 (t, 4H), 3.38 (d, 4H), 4.09 (m, 12H). ^{13}C NMR ($CDCl_3$): d 14.11, 16.31, 16.41, 25.63, 26.03 (JC-P : 135), 27.98, 44.86, 52.43, 60.26, 61.31, 61.41, 63.08, 172.46. (M+H+): 615

General procedure for preparation of corresponding acids: *trans*-cyclohexane-1,2-diamine-*N*,*N'*-acetic-*N*,*N'*-propionic acid 4a and *trans*-cyclohexane-1,2-diamine-*N*,*N'*-acetic-*N*,*N'*-ethylphosphonic acid 4b:

Compound 3a or 3b (1 g) was dissolved in 6N aqueous hydrochloric acid (12ml) and heated to reflux overnight. The refrigerant was removed, and the reaction mixture was kept at 70°C to dryness. An additional aqueous hydrochloric acid 6N (12 ml) was then added, and the solution was heated to dryness. the residue was taken up in MeOH and evaporated under reduced pressure. This step repeated twice gave the corresponding acid as an off-white solid, which was dried under vacuum and kept under nitrogen.

Compound 4a: ^{1}H NMR (D_2O): d 1.25-1.40 (m, 4H), 1.60-2.15 (m, 4H), 2.28 (m, 2H), 2.75 (t, 2H), 2.96 (t, 2H), 3.22 (m, 2H), 3.50-3.90 (m, 4H), 4.15 (s, 1H), 4.28 (s, 1H) ^{13}C NMR ($CDCl_3$): d 25.66, 26.22, 28.87, 31.20, 31.99, 47.25, 54.93, 66.92, 175.03, 176.46 (M-H+): 373

Compound 4b: ^{1}H NMR (D_2O): d 1.05-1.40 (m, 4H), 1.65-2.15 (m, 4H), 2.90-3.25 (m, 6H), 3.45-3.65(m, 2H), 3.70-3.95 (m, 2H). ^{13}C NMR ($CDCl_3$): d 25.66, 26.22, 28.87, 31.20, 31.99, 47.25, 54.93, 66.33, 176.73 (M-H+): 445

***trans*-cyclohexane-1,2-diamine-*N*,*N'*-ethylphosphonic-*N*,*N'*-methylphosphonic acid 4c:** A mixture of compound 4c (0.5 g; 1.12 mmol) and phosphorous acid (0.202 g; 2.46 mmol) in 10 ml of dry toluene was heated to 80°C and stirred for 30 min. PCl_3 (0.22 ml; 2.46 mmol) was then added dropwise, and the reaction mixture was kept at this temperature for 20 hours before being cooled to room temperature. The solvent was discarded and the residual product dissolved in a small volume of water. After filtration, the filtrate was evaporated to give a residue which was purified by precipitation in warm acetone and collected by filtration. The purification step was repeated twice to give 4c which was dried under vacuum and kept under nitrogen (0.460 g; 0.83 mmol; 74%). ^{1}H NMR (D_2O): d 1.15-1.65 (m, 4H), 1.75-2.10 (m, 2H), 2.15-2.40 (m, 6H), 3.00-3.60 (m, 10H). ^{13}C NMR ($CDCl_3$): d (M-H+): 517.

***N*,*N*-*Bis*(ethylacetate)-2-bromoethyl-amine 5:** Bromide 5 was synthesised in our laboratory according the synthesis procedure of Williams and Rapoport with minor modifications concerning the bis N-alkylated ethanolamine synthesis. To a 4°C solution of ethanolamine (6ml; 0.1 mol) in 100 ml of dried acetonitrile was added dropwise ethylbromoacetate (7.4 ml; 66 mmol) over a period of 20

min during which time a large quantity of precipitate formed. The mixture was allowed to stir for 2 hours at this temperature. The white solid was removed by filtration and washed with a small quantity of acetonitrile. The filtrate was concentrated under reduced pressure. The resulting liquid was taken up in $CHCl_3$ (100mL) and washed with water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a liquid which was used directly in the next step (5.9 g; 25.32 mmol; 77%). The dialkylated ethanolamine and Ph_3P (7.72 g; 27.9 mmol) were dissolved in CH_2Cl_2 (100mL). The mixture was cooled in an ice bath and vigorously stirred while NBS (4.96 g; 27.9 mmol) was added in small portions. After the solution was stirred at 0°C for two hours, evaporation of the solvent gave a semisolid which was tritured with ether and the resulting solid was separated by filtration. the filtrate was evaporated to give an oil which was purified by column chromatography (silica gel, CH_3Cl). (6.14 g; 20.7 mmol; 62% overall) ^1H NMR ($CDCl_3$): d 1.26 (m, 6H), 3.15 (t, 2H, J = 7.75 Hz), 3.44 (t, 2H, J = 7.75 Hz), 3.59 (s, 4H), 4.15 (q, 4H). ^{13}C NMR ($CDCl_3$): d (M+H+): 297

***N,N'*-[(2-cyano)eth-1-yl]-N,N'-[*N'',N''-bis*-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine 3d:** To a solution of compound 2d (1 g; 4.54 mmol) and bromide 5 (3 g; 10.13 mmol) in a mixed solvent system (CH_3CN-EtOH, 1:1) was added Na_2CO_3 (1.4 g; 13.20 mmol) and KI (0.75 g; 4.54 mmol). After stirring for 2 days at 70°C, the reaction mixture was filtered and concentrated under reduced pressure. The residue was taken up in $CHCl_3$ (200 ml) and washed with water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow-brown oil. The crude product was purified by column chromatography (silica gel; CH_2Cl_2-EtOH 98:2). The fractions containing pure product were collected and dried to give an limpid oil (1.09 g; 1.68 mmol; 37%).^1H NMR ($CDCl_3$): d 1.13 (m, 4H), 1.27 (t, 12H), 1.73 (m, 2H), 1.86 (m, 2H), 2.30-2.85 (m, 4H+2H), 2.95 (m, 2H), 3.55 (s, 8H), 4.17 (m, 12H). ^{13}C NMR ($CDCl_3$): d 14.25, 18.63, 25.79, 27.24, 47.35, 48.98, 53.78, 55.43, 60.54, 62.64, 119.49, 171.11. (M+H+): 652

***trans*-cyclohexane-1,2-diamine-*N,N'*-propionic-*N,N'*-[*N'',N''-bis*-(2-aminoethyl)]-tetra-acetic acid 4d:** this hexaacid has been prepared as described above for compounds 4a & 4b from 1 g of the ester 3d and two volumes of 20 ml HCl (6N). ^1H NMR (D_2O): 1.20-2.00 (m, 10 H), 2.30 (m, 2H), 2.40-3.00 (m, 4H), 3.10-3.95 (m, 14 H), 4.20 (m, 4H) d ^{13}C NMR (D_2O) : d 26.89, 30.49, 40.99, 52.00, 55.18, 55.47, 58.97, 165.50, 170.38. (M-H+): 547

***N,N'*-(diethylphosphono-methyl)-*trans*-cyclohexane-1,2-diamine 7:** was synthesised in our laboratory according the synthesis procedure of Baily and Burgada with minor modifications. Freshly distilled *trans*-1,2-diaminocyclohexane 1 (3.6 ml, 30 mmol) and diethyl phosphite (7.24 ml, 60 mmol) were dissolved in THF (40ml). The mixture was stirred at reflux and paraformaldehyde (2.8 g, 93 mmol) was added over a 30-min period and the reaction mixture was stirred at reflux for 4 hours. The solvent was evaporated to afford a residue which was taken up in $CHCl_3$. The organic layer was washed with brine (2*100 ml), dried and evaporated to leave a crude oil. A purification by column chromatography (silica gel, CH_2Cl_2-EtOH 96:4) gave 6 (9.2 g, 21.60 mmol, 72%). 6 was then dissolved in MeOH (40 ml) and 35% HCl (15 ml) was added. The mixture was stirred at 50°C overnight. MeOH was removed. the aqueous layer was adjusted to 50 ml with H_2O and then neutralized by $HNaCO_3$. bisphosphonate 7 was extracted by $CHCl_3$. Organic layers were collected, dried and evaporated to give 5.6 g of bisphosphonate 7 (13.52 mmol, 62%, 45% overall). ^1H NMR ($CDCl_3$): 1.10 (m, 2 H), 1.27 (t, 12H), 1.48 (m, 2H), 1.76 (m, 2 H), 2.13 (m, 2H), 2.98 (m, 2H), 3.10 (t, 2H), 3.35 (t, 2H), 4.12 (m, 8H). ^{13}C NMR ($CDCl_3$): d: 16.36, 16.39, 16.45, 16.38, 24,01, 28.23, 39.67 (JP-C = 156 Hz), 60.74, 60.89, 63.16, 63.23, 63.26, 63.33. (M+H+): 415

***N,N'*-(diethylphosphono-methyl)-*N*-[*N'',N''*-*bis*-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine 8:** The mixed ester has been prepared in a mixed solvent system (CH_3CN-H_2O, 1:1) as described above for compound 3d from bisphosphonate 6 (1 g, 2.41 mmol) , Na_2HPO_4 (0.5 g; 3.50 mmol) and 1 equivalent of bromide 5 (0.71 g; 2.41 mmol). After stirring for 2 days at 70°C, the reaction mixture was filtered and concentrated under reduced pressure. The residue was taken up in $CHCl_3$ (200 ml) and washed with water. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure to give a yellow-brown oil witch was used directly in the next step. ^1H NMR: 1.00-1.20 (m, 4 H), 1.25 (t, 6H), 1.31 (t,12H), 1.72 (m, 2H), 2.04 (m, 2H), 2.23 (m, 1 H), 2.70-2.95 (m, 6H), 3.12 (m, 2H), 3.65 (s, 4H), 4.15 (m, 18H).

***N,N'*-(diethylphosphono-methyl)-*N'*-(ethylacetate)-*N*-[*N'',N''*-*bis*-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine 9:** The tetraester is prepared as described above for compounds 3a & 3b from compound 8,

Na_2CO_3, KI and ethylbromoacetate (1 equivalent). Purification by chromatography (SiO_2) gave a oil.

N,N'-(diethylphosphono-methyl)-*N,N'*-[*N'',N''*-*bis*-(ethylacetate-2-aminoethyl)]-*trans*-cyclohexane-1,2-diamine **12**: The mixed ester is prepared as described above for compound 3d from bisphosphonate 7 (1g, 2.41 mmol), Na_2HPO_4 (1 g, 7.04 mmol) and 2 equivalents of bromide 5 1.80 g, 6.08 mmol). Purification by chromatography (SiO_2 (CH_2Cl_2-MeOH 95 : 5) gave a pale yellow oil (0.59 g, 0.70 mmol, 29%). ^1H NMR: 1.16 (m, 4H), 1.25 (t, 12H), 1.33 (t, 12H), 1.69 (m, 2H), 1.86 (m, 2H + 2H), 2.70-3.50 (m, 8H + 2H + 2H), 3.57 (s, 8H), 4.12 (m, 16H). (M+H+): 847

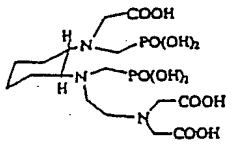
trans-cyclohexane-1,2-diamine-*N,N'*-methylphosphonic-*N*-*acetic*-,*N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-tetra-acetic acid **10** & *trans*-cyclohexane-1,2-diamine-*N,N'*-methylphosphonic-*N,N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-tetra-acetic acid **13** : Those compounds are prepared as described above for compound 4a, 4b and 4d.

Compound 13: ^1H NMR (D_2O): 1.26 (m, 2 H), 1.45 (m, 2H), 1.75-2.00 (m, 4H), 2.80 (m, 2H), 3.00-3.70 (m, 12H), 4.10 (br s, 8H). (M-H+): 619

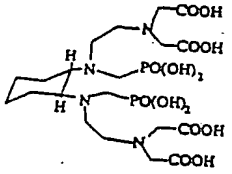
trans-cyclohexane-1,2-diamine-*N,N,N'*-methylphosphonic-*N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-di-methylphosphonic acid **11** & *trans*-cyclohexane-1,2-diamine-*N,N'*-methylphosphonic-*N,N'*-[*N'',N''*-*bis*-(2-aminoethyl)]-tetra-methylphosphonic acid **14** : Those compounds are prepared as described above for compound 4c.

153Sm complexation studies

Complexation studies with Samarium 153 were performed on the two following chelating agents 10 (AL 247) and 13 (AL 245)



10



13

Radiochemistry purity was measured on ITLC-SG chromatographic profiles. Radioactivity was quantified using a Phosphorimager 445SI apparatus.

Samarium 153 was furnished under $^{153}SmCl_3$ form in HCl 0,04N with a 5,2 GBq/ml volumic activity and a 40 GBq/mg specific activity.

They were tested for their complexation properties by using an excess of 10 to 50 equivalents of chelating agent.

Competition studies were performed against EDTMP according to the following method :

- first step : 50 equivalents of one of the chelating agents and a fixed amount of 153Sm were incubated at 37°C during 3 hours in order to form the 153Sm-CA complex (CA = Chelating Agent),

- second step : 50 equivalents of EDTMP were added to the previous solution and kept 3h at 37°C in order to measure the decomplexation. Another measure was performed 72h after to ensure a complete decomplexation possibility.

Results :

	% of non decomplexed 153Sm-CA	
chelating agent	after 3h	after 72h
10 (AL 247)	100	67
13 (AL 245)	100	100

AL 247 and AL 245 present very good complexation properties for 153Sm and in any cases better than EDTMP

Furthermore, stability was performed on AL 245 in human serum media at 37°C at different time and showed no loss of 153Sm from AL 245 at either 24, 48, 72 and 96h.

References

- T. Baily and R. Burgada, *Phosphorus, Sulfur and Silicon.*, 1995, **101**, 131.

- M.Bardies, P. Thedrez, J.F. Gestin, B.M. Marcille, D. Guerreau, A. Faivre-Chauvet, M. Mahé, C. Sai-Maurel and J.F. Chatal, *Int. J. Cancer*, 1992, **50**, 984.

- R. J. Bergeron, P.S. Burton, K.A. McGovern aaaand S.J. Kline, *Synthesis*, 1981, 732

- J-F. Gestin, E. Benoist, A. Loussouarn, A.K. Mishra, A. Faivre-Chauvet and J-F. Chatal, *New J. of Chem.*, 1997, **21**, 1021.

- W. F. Goeckeler, B. Edwards,W.A. Volkert, R.A. Holmes, J. Simon and D. Wilson, *J. Nucl. Med.*, 1987, **28**, 495.

- F. Krüger and L. Bauer, *Chem.Ztg.*, 1972, **36**, 691.

- A. Loussouarn, M. Duflos, E. Benoist, J-F. Chatal, G. Le Baut and J-F. Gestin, *J. Chem. Soc. Perkin Trans., 1998,* **1**, 237.

- C.F. Meares, M.J. Mc Call, D.T. Reardan, D.A. Goodwin, C.I. Diamanti and M. McTigue, *Anal. Chem.*, 1984, **142**, 68.

- R.C. Mease, S.C. Srivastava, G.E. Meinken, J-F. Gestin and Z. Steplewski, *J. Nucl. Med.*, 1990, **31**, 896.

- P. L. Ornstein, J. M. Schaus, J. W. Chambers, D. L. Huser, J. D. Leander, D. T. Wong, J. W. Paschal, N. D. Jones and J. B. Deeter, *J. Med. Chem.* 1989, **32**, 827.

- D.Parker, *Chem. Soc. Review,* 1990, **19**, 271.

- P.A. Schubiger, R. Alberto and A. Smith, *Bioconjugate Chem.*, 1996, **7**, 165.

- R. Stein, D. M. Goldenderg, S. R. Thorpe, A. Basu and M. J. Mattes, *Cancer Research*, 1995, **55**, 3132.

- M. Studer and C.F. Meares, *Bioconjugate Chem.*, 1992, **3**, 420.

- M. A. Williams and H. Rapoport, *J. Org. Chem.*, 1994, **59**, 3616.